

# Ultrastructural Study of the Peritoneum in Patients on Continuous Ambulatory Peritoneal Dialysis

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Twenty peritoneal specimens, collected from 19 patients at the insertion or removal of the catheter for continuous ambulatory peritoneal dialysis (CAPD), were examined by light microscopy (LM) and transmission and scanning electron microscopy (TEM and SEM). During long-term CAPD, the peritoneal tissue showed an absence of mesothelial cells and a fibrous thickening by proliferation of degenerative collagen fibers. Ultrastructural examination by SEM revealed that the surface of the peritoneum with mesothelial denudation was covered by a continuous sheet of homogeneous material (a membrane structure) in patients in an the early stage of peritonitis. In cases in the advanced stage, the membrane structure covered the irregular collagen bundles, which occasionally showed through breaks in the membrane-like structure. Vascular alterations characterized by the hyalinous degeneration of media, the thickening of the basement membrane in small vasculature, and lymphatic dilatation were observed by TEM in cases of sclerosing peritonitis. Our results suggest that the pathological changes of the peritoneal surface and peripheral blood and lymphatic circulatory impairment may be related to ultrafiltration failure and the progression of pathological process during CAPD.

**Key words :** CAPD, Mesothelium, Peritoneum, Sclerosing peritonitis, Ultrafiltration, Electron microscopy

## Introduction and History

Ultrafiltration failure and sclerosing peritonitis have been recognized as important complications accompanying the increased use of long-term continuous ambulatory peritoneal dialysis (CAPD) [1,2,3]. In the peritoneum of patients treated by CAPD, Dobbie et al. noted disappearance of microvilli in mesothelial cells, the sloughing of mesothelial cells, and the proliferation of connective tissue [4,5]. However, the relationship between the histological findings and the effects of the dialysis has not been adequately explored, and little detailed ultrastructural study of peritoneal tissue in cases of sclerosing peritonitis has been reported. For this study, we examined the detailed morphological course of sclerosing peritonitis and correlated of pathological changes and filtration function, focusing on ultrastructural morphology, in the peritoneum of CAPD patients.

## Materials and Methods

### Patients

Twenty peritoneal specimens were collected from 19

Table 1. Clinical data of the patients with CAPD therapy

Case No.	Age	Sex	Duration of CAPD (month)	History of Peritonitis	Cause of CRF
Group of patients with ultrafiltration failure					
1	44	M	84	2	CGN
2	44	F	48	2	CGN
3	44	M	53	1	CGN
4	70	M	48	1	DM
Group of patients without ultrafiltration failure					
1	75	M	4	1	CGN
2	50	M	54	3	CGN
3	2	F	6	2	CGN
4	54	M	12	1	CGN
5	58	M	3	0	CGN
6	24	M	36	0	CGN
Group of patients at the start of CAPD					
9 patients (8 males and 1 female)					
mean age : year(range : 33-64)					

patients (16 males and 3 females), who were treated for endstage renal diseases by CAPD. Five specimens were obtained from 4 patients in whom CAPD therapy was interrupted due to ultrafiltration failure. CAPD therapy was effective for ultrafiltration in 6 patients, but the catheter was removed in 3 of 6 because of active peritonitis or gastric operation. Nine peritoneal specimens obtained before the start of the CAPD were used as control. The patients and clinical data are summarized in Table 1. The mean age of the patients with ultrafiltration failure was 44.5 years (range : 41-62 years), while that of the patients without ultrafiltration failure was 42 years (range : 2-72 years). The mean duration of CAPD was 4.6 years (range: 4-7 years) and 1.6 years (range : 4 months-7 years) in patients with and without ultrafiltration failure. There was no difference in the frequency of peritonitis with respect to ultrafiltration failure.

#### Tissue preparation

The peritoneal specimens were obtained from the parietal peritoneum at the insertion or removal of the catheter for CAPD. The specimens, measuring approximately 5 x 4 mm, were immediately fixed after excision. Specimens to be examined by LM were fixed in 10% formaldehyde, embedded in paraffin and stained with hematoxylin and eosin, periodic acid-Schiff reagent, Masson trichrome, and von Gieson elastica methods. Specimens to be examined by SEM were prepared by cutting small pieces of tissue into small cubes and fixing them in buffered 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) at 4 °C. Thereafter, the specimens were immersed in 3% tannic acid in a 0.1 M phosphate buffer (pH 7.4) at 4 °C, then post-fixed with 2% osmium tetroxide at 4 °C and dehydrated in a graded ethanol series before being placed in t-butyl alcohol. The specimens were dried by the t-butyl alcohol freeze-dry method in an evacuator (ID-2, RMC, EIKO Co.

Japan), coated with gold, and observed by SEM (JEM35c/LaB6) at an accelerating voltage of 15 kV.

The specimens to be examined by TEM were fixed in buffered 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) at 4 °C, post-fixed in 2.5% osmium tetroxide at 4 °C, dehydrated in a graded ethanol series, and embedded in epon. Then ultrathin sections double-stained with 2% uranyl acetate and lead citrate were examined by TEM (JEM-1200) at an accelerating voltage of 60 kV.

## Results

### *Peritoneum before the start of CAPD*

In every case, before the start of CAPD, the peritoneum was lined by a layer of mesothelial cells associated with the thin collagen tissue underneath the mesothelium (Figure 1). Small blood vessels were found scattered among the collagen fibers. Examination of the peritoneal tissue by SEM revealed that the surface of the peritoneum was lined with mesothelial cells measuring 10 μm x 6 μm, with microvilli (Figures 2 and 3). Examination of the peritoneal tissue by TEM revealed that the peritoneum was lined with a layer of mesothelial cells, 0.7 to 1.8 μm long and 0.5 to 1.8 μm in thickness. The mesothelial cells contained cigar-shaped nuclei and many organelle, and the cell membranes at the site of abdominal cavity had microvilli. No pathological findings were observed in the peritoneal tissue at the start of CAPD by either light or electron microscopy.

### *CAPD patients without ultrafiltration failure*

In all the patients of this group, CAPD therapy was effective for ultrafiltration. However, the peritoneal tissue taken showed various histologic features corre-

**Table 2.** Pathological Findings of patients with CAPD therapy

	Patients with ultrafiltration failure	Patients without ultrafiltration failure	Patients at start of CAPD
Peritoneal fibrosis	+++	-~++	-
inflammatory infiltrates	+~+++	-~+++	-
Peritoneal surface			
mesothelial denudation	+++ (diffuse)	-~+ (focal)	-
fibrinous exudation	+	-~±	-
break of membrane	+	-~±	-
Blood vessel			
wall thickening	+~+++	-~±	-
hyalinous degeneration	+	-	-
thickening of			
basement membrane	+	-~±	-
Lymphatic dilatation	+	-	-

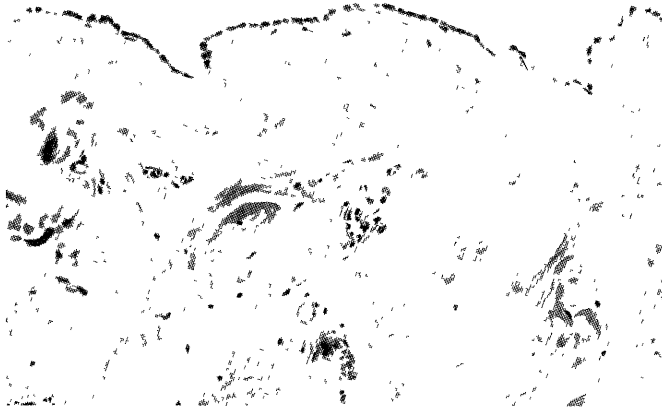


Fig. 1. Light microscopy of the peritoneum before CAPD. The peritoneum is covered with a monolayer of regularly arranged mesothelial cells with scattered collagen fibers, stained with hematoxylin and eosin, x 120.

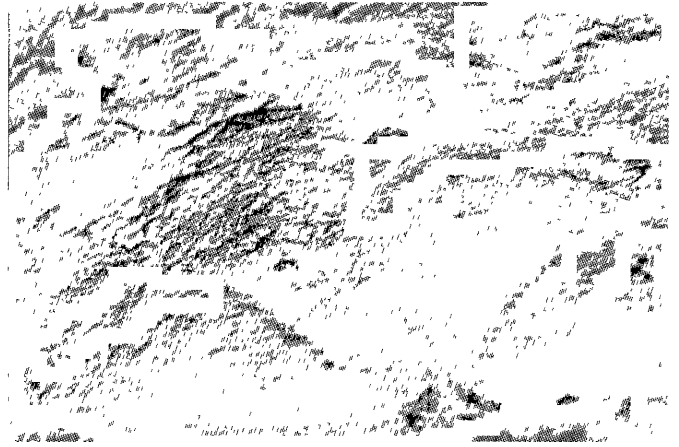


Fig. 2. Peritoneum before start of CAPD. The surface is covered by slightly elevated mesothelial cells, measuring about 10 μm x 6 μm. SEM, x 7500

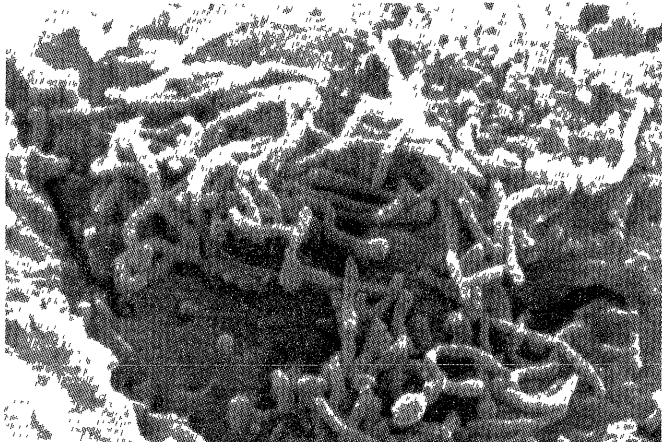


Fig. 3. Surface of mesothelial cells with numerous microvilli. SEM, x 36000

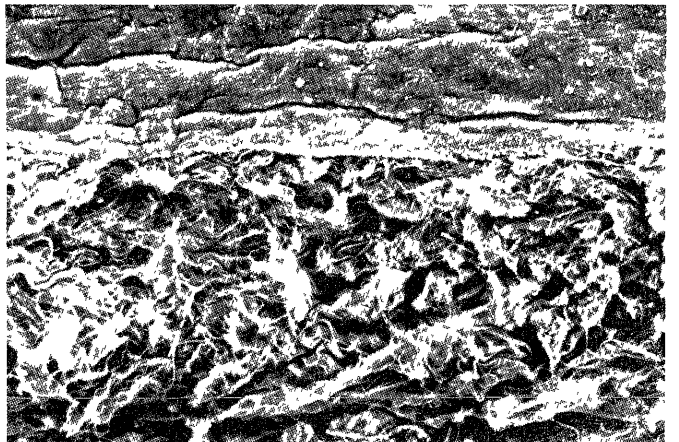


Fig. 4. Mild peritoneal fibrosis. There was irregular proliferation of collagen fibrils beneath the mesothelial cell layer. Mesothelial cells were irregularly arranged, and their shape and size were not uniform. Note membrane structure between mesothelial cell layer and collagen fibrils. SEM, x 1800

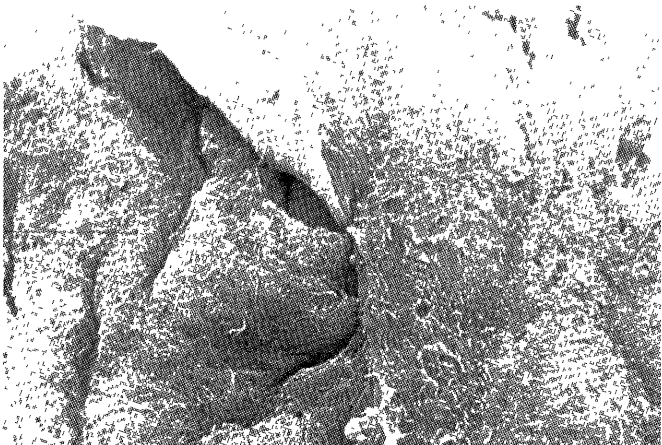


Fig. 5. Peritoneum is focally covered by mesothelial cells (right side). Surface at the left side is denuded and shows a creased sheet of homogeneous material (membrane structure). SEM, x 7500

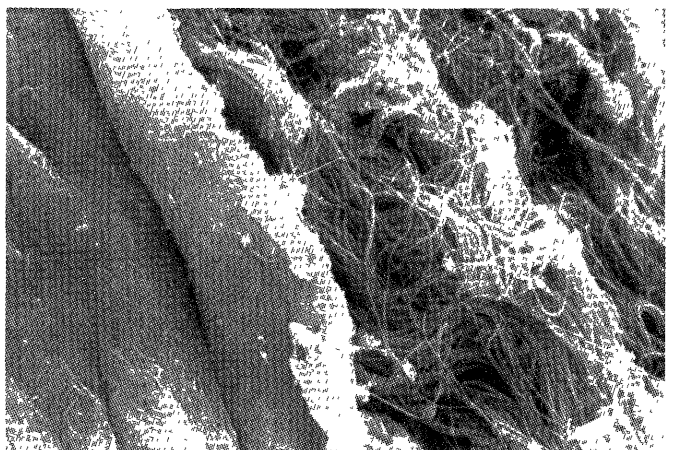


Fig. 6. Dense proliferation of bundle-shaped fibers is observed (right side) below the membrane-like structure containing fine fibrils (left side). SEM, x 6500



Fig. 7. Peritoneum of the patient with mild fibrosis after 4 months of CAPD. Although peritonitis occurred during CAPD, ultrafiltration function was preserved. Mesothelial cells were sloughed off, and the surface is covered by a continuous sheet of a homogeneous membrane structure with scattered small depressions. SEM, x 1200

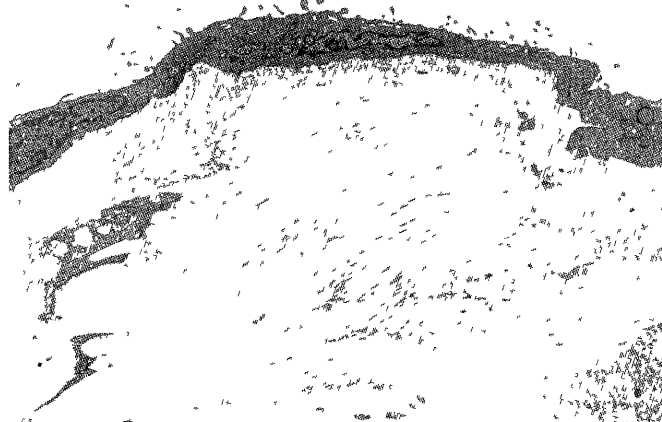


Fig. 8. Peritoneum with fibrosis. The surface is lined with a layer of mesothelial cells possessing microvilli, below which is a layer of proliferated collagen fibers. Cellular infiltration was rarely observed. TEM, x 4000

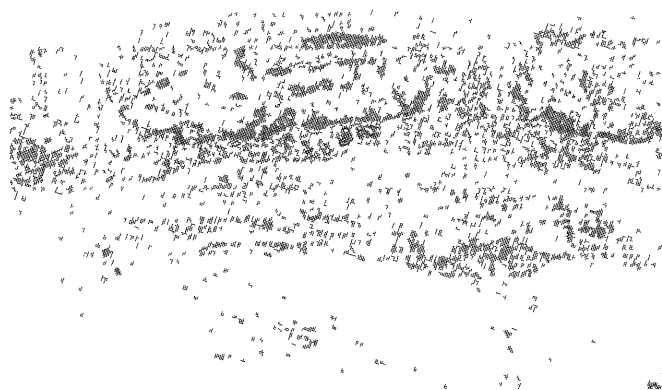


Fig. 9. Peritoneum. Below mesothelial cells is a layer composed of short fibrils embedded in submesothelial matrix. At the deeper region lies a dense layer of proliferated bundle-shaped collagen fibrils. TEM, x 10000

sponding to a variety of clinical conditions (Table 2). Peritoneal fibrosis was observed in two patients in a mild to moderate degree, though mesothelial cells were preserved, both patients had a clinical history of recurrent peritonitis. Another patient showed active inflammatory infiltrates in a thickened peritoneum, associated with focal mesothelial detachment; perivascular inflammatory cell infiltration was seen in this case. The remaining three patients in this group had no histological abnormalities in the peritoneum. No apparent thickening of vascular walls was seen in any patient of this group.

Examination of tissue by SEM revealed that after a short period of CAPD the peritoneum had become covered with mesothelial cells possessing no pathological changes. In the cases with a thickened peritoneum, an irregular proliferation of collagen fibers was found beneath the mesothelial cell layer (Figure 4). Mesothelial cells were irregularly arranged and not uniform in shape and size. Patients with mild or moderate peritoneal fibrosis, the mesothelial cells were partly detached, and at these sites the denuded surface was covered by a continuous sheet of homogeneous material (membrane structure) (Figure 5). This membrane structure, which was somewhat creased, had a formed mesh-like appearance and contained fused fine fibrils (Figures 5 and 6). Compact bundle-shaped fibrils were seen beneath it (Figure 6). That is, the membrane structure was present between the mesothelial cell layer and the collagen fibril layer. Although the membrane structure was creased in many instances, in a few specimens a smooth continuous homogeneous membrane structure covered the surface of the peritoneum. Figure 7 shows diffuse detachment of mesothelial cells and a smooth membrane structure with depressed pits resembling small apertures.

Examination of the thickened peritoneal tissue by TEM revealed two different layers of collagen tissue under the mesothelial cells (Figure 8). Just beneath the mesothelial cells was a thin layer composed of short fibrils embedded in an amorphous extracellular matrix (Figure 9). This layer may correspond to the continuous homogeneous membrane structure observed by SEM. The deep part of the submesothelium was composed of densely packed bundles of collagen fibrils occasionally associated with cellular components, including a few fibroblasts.

#### *CAPD patients with ultrafiltration failure*

In the samples taken when the catheter was removed because of ultrafiltration failure, the mesothelial cells were mostly detached, and the peritoneal tissue was markedly thickened by the irregular proliferation of collagen fibers with occasional hyalinous changes, presenting the picture of sclerosing peritonitis (Figure 10). Thickened walls were observed in the small arteries embedded in collagenous tissue (Figure 11).

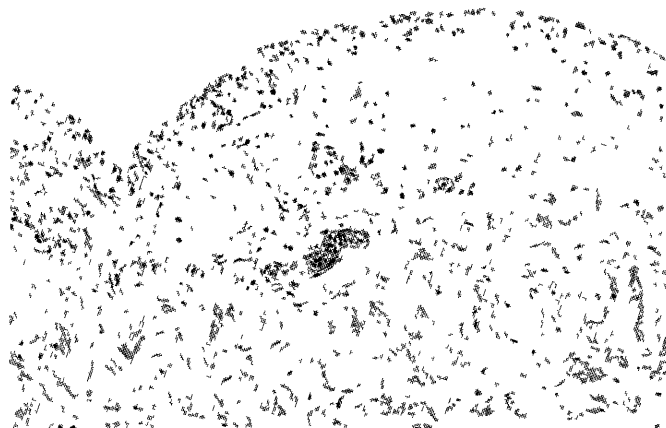


Fig 10. Sclerosing peritonitis with ultrafiltration failure There were loss of mesothelial cells, extensive interstitial fibrosis, and scattered inflammatory cells hematoxylin eosin, x 120

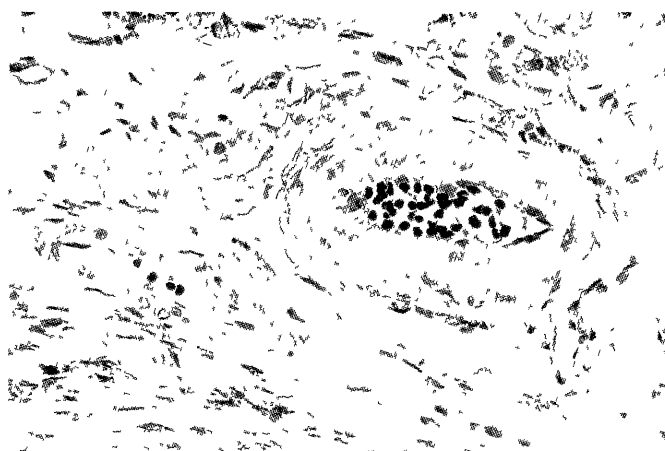


Fig. 11 A small artery with thickened wall surrounded by collagenous tissue hematoxylen and eosin, x 250

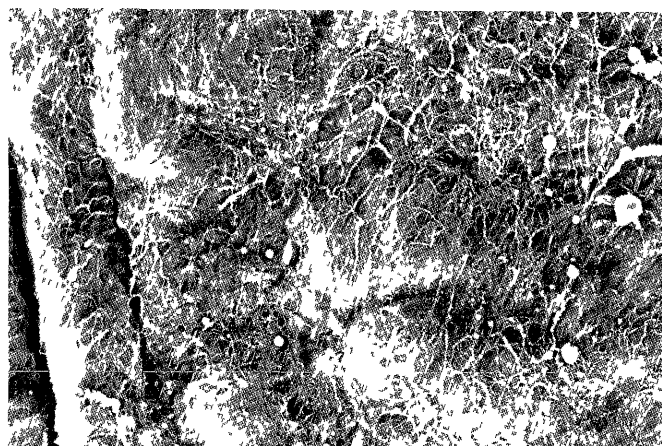


Fig. 12 Sclerosing peritonitis with ultrafiltration failure Peritoneal surface showed loss of mesothelial cell, fibrin exudation, and leukocytic infiltration SEM, x 2800

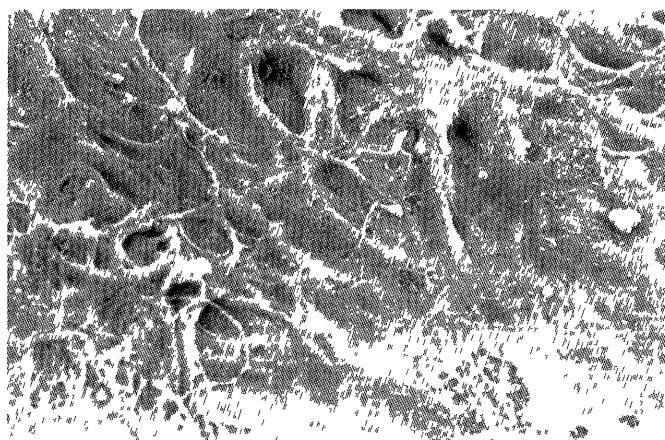


Fig. 13. Sclerosing peritonitis with ultrafiltration failure Mesothelial cells were diffusely detached an and increased number of collagen fibers formed an irregular peritoneum surface Bundles of fibrils were observed through breaks in membrane structure SEM, x 2000

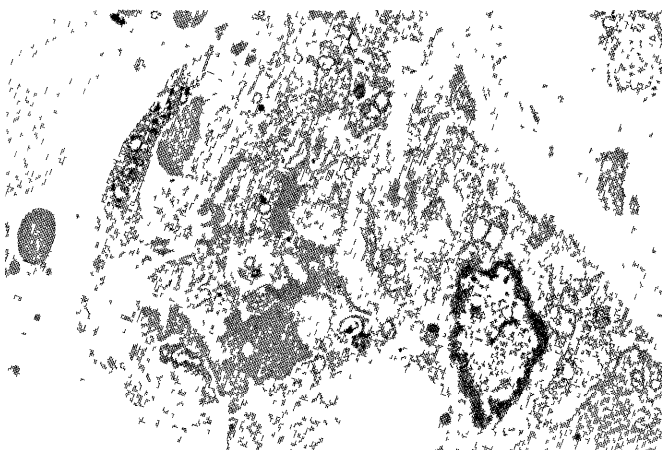


Fig. 14. Sclerosing peritonitis with ultrafiltration failure A small artery with fibrinous degeneration of media TEM, x 7000

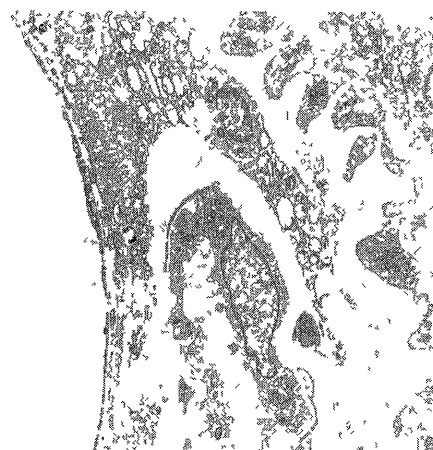


Fig. 15. Peritoneum with loss of ultrafiltration Dilation of the lymphatic vessels (left side) and a capillary vessel with laminated thickening of basement membrane is observed TEM, x 6000

Examination by SEM of the peritoneal tissue of the patients with ultrafiltration failure showed that the surface of the peritoneum was severely irregular, with loss of mesothelial cells, fibrin exudation, and leukocytic infiltration (Figures 12 and 13). Occasionally, hyperplastic bundles of collagen fibrils could be seen through the ruptured membrane structure (Figure 13). Such ultrastructural features appear in the advanced stage of sclerosing peritonitis.

Examination by TEM revealed marked proliferation of degenerative collagen fibrils in the peritoneum for the patients in this group. The pathological changes in the vasculature were characterized by fibrinous degeneration of the media and lamellar thickening of the basement membrane in the small arteries and capillaries (Figures 14 and 15). We also observed dilated lymphatic vessels in the peritoneum at the time of removal of the CAPD catheter. (Figure 15).

## Discussion

Since Popovich et al. introduced the concept of it in 1976, CAPD has been widely used in many countries [1]. However, long-term CAPD has been known to be complicated by ultrafiltration failure [2] and late-stage sclerosing peritonitis [3]. Dobbie et al. described the morphology and function of the peritoneum in normal controls and CAPD patients [4, 5, 6]. They observed that the peritoneal specimens obtained during CAPD showed a loss of mesothelial cells and a proliferation of connective tissue. In the present study, we examined 20 specimens from the parietal peritoneum in CAPD patients by light and electron microscopy. We found histological features of the peritoneal tissue before the start of CAPD were consistent with those of the normal peritoneum [4,5]. Mesothelial cell detachment started at an early stage of peritonitis in our patients. Schneble et al. mentioned that the loss of the mesothelial cell layer appeared to be related to the duration of CAPD treatment [7]. Dobbie et al. also stated the possibility that replacement of the normal loose subserosal stroma by dense collagen might seriously restrict the recruitment and mobility of mesothelial stem cells. [6]. Watters et al. reported that a remnant of the mesothelial basement membrane and absence of connective tissue injury was necessary for mesothelial regeneration in experiments with rats [8]. We observed no mesothelial regeneration during the course of the cases of peritonitis in our study.

Several reports noted vascular alterations in the peritoneum of CAPD patients [9, 10]. Di Paolo et al. observed the replication of the basement membrane of the peritoneal capillaries in both diabetics and nondiabetics. They mentioned that CAPD treatment was an experimental model for microangiopathy in humans [9]. Honda et

al. also described vascular changes in CAPD patients with filtration failure, [10] specifically, severe fibrosis and hyalinization of the media of venules. They suggested that these vascular changes might be caused by certain toxic factors, such as a high osmolar dialysate or low pH of dialysate. In our study, vascular changes were characterized by thickening of the basement membrane and fibrinous degeneration of small striae vasculares in the peritoneum of the CAPD patients with filtration loss. These changes in the blood vessels might be induced by the use of high osmolar dialysate containing glucose. However, additional factors might actuate the vascular changes, because these changes did not appear in all the patients treated with long-term CAPD.

In addition to vascular changes in the small blood vessels, we also observed dilated lymphatic vessels in the thickened peritoneum. The function of lymphatic drainage may be associated with water clearance capacity in the abdominal cavity. Constant lymphatic absorption from the peritoneal surface to the venous system has a great influence on water clearance during peritoneal dialysis. Daugirdas et al. studied the volume of lymphatic drainage but did not arrive at a constant value [11]. The route of lymphatic drainage in the peritoneum is well known. Water, protein, and blood cell components are excreted to peritoneal cavity through the lymphatic stomata opening onto the surface of the peritoneum. Lymphatic stomata are distributed in the mesentery and omentum, and lymphatic drainage connects the venous system via the thoracic duct. They are also found opening into the diaphragm. An increase in intraperitoneal pressure is known to increase the lymphatic absorption [12, 13]. Our observations of dilated lymphatic vessels and stoma-like apertures in the patients with peritonitis suggests that ultrafiltration impairment may reflect lymphatic flow stagnation.

To summarize the morphological course of the peritoneal changes: mesothelial cell alterations characterized by focal denudation and degeneration may begin to appear within 6 months from the start of CAPD. In the absence of infectious peritonitis, this process may continue for a long time without ultrafiltration failure. Reoccurrence of peritonitis may promote diffuse detachment of the mesothelial cells and peritoneal fibrosis with proliferation and degeneration of collagen fibrils. At this stage, the surface of the peritoneum may be covered with a membrane structure. As this membrane structure is not the basement membrane of the mesothelium, no regeneration of the mesothelial cell can occur. The significance or function of the membrane structure is not known. Since the membrane structure is composed of a thin layer of accumulated collagen fibrils embedded within a matrix, the structure seems to protect collagen fibrils from direct exposure to the abdominal cavity. Peritoneal fibrosis at this stage, unaccompanied by ultrafiltration failure, might

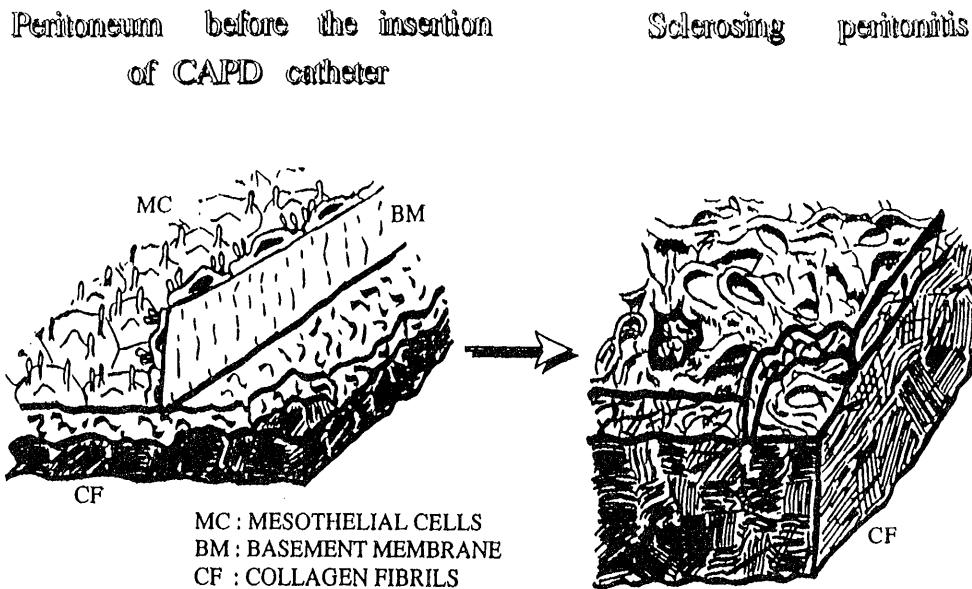


Fig. 16. Schema of the parietal peritoneum from a patient on CAPD.

be an early stage of sclerosing peritonitis. Later, an increase in collagen fibers formed the irregular peritoneal surface, with an occasional rupture of the covering membrane structure. Also present were pathological changes of vasculature. In this advanced stage of sclerosing peritonitis, the ultrafiltration function of the peritoneum may be severely impaired (Figure 16).

In this study, we showed the detailed morphological course of sclerosing peritonitis in CAPD patients, focusing on ultrastructural changes. We conclude that the alteration of the peritoneal surface, including the detachment of mesothelial cells and the rupture of the membrane-like sheet with exposure of the collagen fibrils, and peripheral blood and lymphatic circulatory impairment, may be related to ultrafiltration failure and the progression of a pathological process during CAPD.

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### References

- 1) Popovich RP, Moncrief JW, Decherd JF, et al. The definition of a novel potable/wearable equilibrium dialysis technique. *Trans Am Soc Artif Intern Organs*, 5 : 64A, 1976.
- 2) Verger C, Brunsvicg O, Le Charpentier Y, et al. Structural and ultrastructural peritoneal membrane changes and permeability alterations during continuous ambulatory peritoneal dialysis. *Proc EDTA*, 18 : 199-205, 1981.
- 3) Oreopoulos DG, Khanna R, Wu G. Sclerosing obstructive peritonitis after CAPD. *Lancet*, ii : 409, 1983.
- 4) Dobbie JW, Zaki MA, Wilson, LS. Ultrastructural studies on the peritoneum with special references to chronic ambulatory dialysis. *Scott Med J*, 26 : 213-223, 1981.
- 5) Dobbie JW, Lloyd JK, Gall CA. Categorization of ultrastructural changes in peritoneal, stroma and blood vessels in uremia in CAPD patients. In *Advances in Peritoneal Dialysis* (Baxter R and D Europeeds, Nivelles, Belgium), pp. 3-12, 6, 1990.
- 6) Dobbie JW. New concepts in molecular biology and ultrastructural pathology of the peritoneum : their significance for peritoneal dialysis. *Am J Kidney Dis*, 15 : 97-109, 1990.
- 7) Schneble F, Bonzel KE, Waldherr R, et al. Peritoneal morphology in children treated by continuous ambulatory peritoneal dialysis. *Pediatr Nephrol*, 6 : 542-546, 1992.
- 8) Watters WB, Buck RC. Scanning electron microscopy on mesothelial regeneration in the rat. *Lab Invest*, 26 : 604-609, 1972.
- 9) Di Paolo N, Sacchi G. Peritoneal vascular changes in cutaneous ambulatory peritoneal dialysis. An in vivo model for the study of diabetic microangiopathy. *Perit Dial Int*, 9 : 41-45, 1989.
- 10) Honda K, Nitta K, Horita S, et al. Morphological changes in the peritoneal vasculature of patients on CAPD with ultrafiltration failure. *Nephron*, 72 : 171-176, 1996.
- 11) Daugirdas JT, Ing TS, Gandhi VC, et al. Kinetics of peritoneal absorption in patients with chronic renal failure. *J Lab Clin Med*, 95 : 351-361, 1980.
- 12) Abernethy NJ, Chin W, Hay JB, et al. Lymphatic drainage of the peritoneal cavity in sheep. *Am J physiol*, 260 : F353-F358, 1991.
- 13) Krediet RT, Boeschoten EW, Struijk, D.G. Struijk, L. Arisz. Difference in the peritoneal transport of water, solute and proteins between dialysis with two- and with three-liter exchange. *Nephrol Dial Transplant*, 2 : 198-204, 1988.